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# Applications of a Novel Biasing Potential to Study DNA Translocation and DNA Base Flipping

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DNA transcription, replication, and damage repair usually involve DNA-protein interactions and structural distortion of the DNA duplex by various enzymes. For example, during DNA metabolism, DNA helicases have been shown to separate duplex DNA into individual strands by translocating along single stranded DNA (ssDNA) while hydrolyzing ATP<sup>1,2</sup>. Alternatively, various enzymes employ a base-flipping mechanism to tackle DNA repair<sup>3</sup>. Experimental studies have demonstrated that DNA translocation and flipping of DNA base pairs typically occurs on the millisecond or longer timescale<sup>4,5</sup>. However, current computational methods are limited to the nanosecond timescale. Thus, external restraints are often employed to enhance sampling in these low probability regions of phase space. While flipping of individual bases using different restraints has been well established<sup>3</sup>, computational studies related to DNA translocation with respect to proteins is, to the best of our knowledge, slowly emerging<sup>6</sup>. In this study, umbrella sampling with a novel center-of-mass projection onto a predefined path reaction coordinate was utilized to study DNA translocation in the context of the transcription factor, E2F-DP, protein-DNA complex using implicit solvent.

## 1 Introduction

Protein-DNA interactions involving DNA base flipping and DNA translocation are vital for the proper functioning and survival of a cell. However, studying the mechanisms and energetics associated with these processes using straight molecular dynamics simulations are often unfeasible within the available computer time. The novel biasing potential presented below was used to study DNA translocation (in the presence of a DNA-binding protein) and is currently being applied to DNA base flipping (in the absence of a protein) though its function can be extended beyond these applications.

## 2 Development of New Biasing Potential

As a first step to understanding DNA translocation (and DNA base flipping), a new biasing potential,  $U_{res}$ , was developed and can be described as the projection of one or more center of mass (COM) onto a well understood path reaction coordinate. This harmonic potential (added to CHARMM<sup>7</sup>)

$$U_{res} = k(t' - t_{initial} - t_0)^2 \quad (1)$$

can be expressed in terms of its initial COM projection onto the path  $t_{initial}$ , its COM projection onto the path at a given timestep  $t'$ , its equilibrium COM projection value  $t_0$  (relative to  $t_{initial}$ ), and its force constant  $k$ . To elaborate, for a given well-behaved path

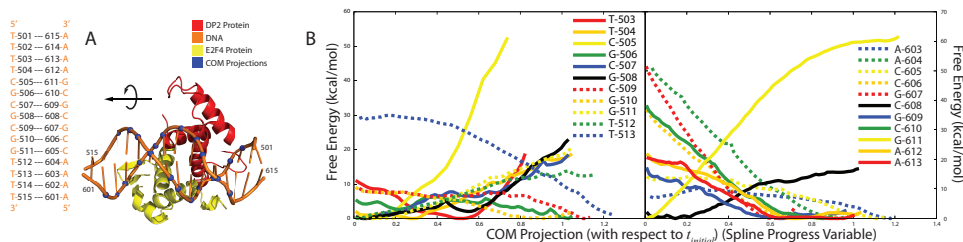


Figure 1. A. COM projection of select nucleotides onto a defined path. The translocation direction is shown in black arrows. B. Free energy profiles of each individual nucleotide. The units along the horizontal axis roughly corresponds to 3.8 angstroms (the rise in DNA).

represented by a set of points, cubic spline interpolation is used to reconstruct a piecewise smooth curve and the COM of interest is projected onto this curve. Since the projection of any point,  $P(x, y, z)$ , onto a cubic spline involves solving the roots of a quintic equation (which has no closed form solution), the method was simplified by projecting the COM onto a tangent line (with the assumption that this tangent is sufficiently close to the real tangent and remains constant within a given simulation window). Once the projection is approximated the umbrella potential is applied and is repeated for subsequent simulation windows by modifying  $t_0$  at the start of each window.

### 3 Translocation of E2F-DP-DNA Complex

To study DNA translocation, the DNA-bound crystal structure of a heterodimeric transcription factor, E2F-DP, (PDBID: 1CF7<sup>9</sup>) was used where the path reaction coordinate was chosen as the DNA backbone. With the exception of the two terminal nucleotides from each DNA strand, the COM for each nucleotide was projected onto its own DNA backbone (Fig. 1A). After equilibration, the molecular dynamics simulations were carried out at 300 K using GBMV implicit solvent and free energy profiles were generated using the weighted histogram analysis method (WHAM)<sup>8</sup>. To facilitate DNA translocation by one base pair, all values of  $t_0$  were uniformly adjusted over the course of 20 simulation windows away from DP and closer towards E2F, carrying out 50 ps of equilibration and at least 50 ps of sampling for each umbrella (while the path reaction coordinate remains unchanged).

The results obtained from WHAM analysis reveal important interactions between the amino terminus of the E2F protein and the DNA. With the exception of Cyt505 (and its hydrogen bonding partner, Gua611), the free energy profiles for each individual base (Fig. 1C) show general fluctuations in the relative free energies which can be associated with the breaking of hydrogen bonds between the protein-DNA complex. However, as Cyt505 moves along the reaction coordinate it encounters a key protein residue, Arg17 (from the amino-terminal of E2F), that is situated deep into the minor groove. According to Jordan et al, this conserved arginine appears to be important as its deletion abolishes DNA binding though its structural role is unclear<sup>10</sup>. Our findings suggest that once the E2F-DP dimer recognizes and binds to the central CGCGCG sequence, Arg17 plays an integral part in hindering DNA translocation.

## 4 DNA Base Flipping

For DNA base flipping, the COM of the central cytosine base (in GTCAGCGCATGG) was projected onto the path reaction coordinate which was chosen as the perimeter of a circle lying in the plane of the nitrogenous base and centered about the C3' atom of the residue of interest. To induce base opening,  $t_0$  was adjusted over the course of 160 simulation windows towards the major and minor groove, respectively, carrying out 50 ps of equilibration and 200 ps of sampling for each individual window. The results to date are in good structural agreement with the current knowledge<sup>3</sup>.

## 5 Acknowledgments

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